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Pollinator attraction in *Cornus capitata* (Cornaceae): the relative role of visual and olfactory cues

Bo Song[1], *Yang Niu*[1], *Jürg Stöcklin*[2], *Gao Chen*[3], *De-Li Peng*[1], *Yong-Qian Gao*[4] *and Hang Sun*[1,*]

[1] *Key Laboratory for Plant Diversity and Biogeography of East Asian, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Kunming 650201, Yunnan, China*
[2] *Section of Plant Ecology, Institute of Botany, University of Basel, Schönbeinstrasse 6, Basel 4056, Switzerland*
[3] *Kunming Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Kunming 650201, Yunnan, China*
[4] *Department of Ecology and Environmental Engineering, Yunnan Forestry Technological College, 1 Chuanjin Road, Kunming 650224, Yunnan, China*
*Correspondence address. Key Laboratory for Plant Diversity and Biogeography of East Asian, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Kunming 650201, Yunnan, China. Tel/Fax: +86-871-65215002; E-mail: hsun@mail.kib.ac.cn

Abstract

Aims
It is generally accepted that visual displays and floral scent play important roles in communication between flowering plants and their pollinators. However, the relative role of visual and olfactory cues in pollinator attraction is largely unknown. In this study, we determined the roles of both types of cue in attracting pollinators to *Cornus capitata*, a medium sized tree with each capitulum surrounded by four large, white, petaloid bracts.

Methods
Pollinator observations and pollination experiments were conducted in a natural population; the inflorescences' visual and olfactory signals were characterized by spectral and chemical analyses; the responses of pollinators to visual and olfactory cues were tested using dual-choice behavioural bioassays; the relative roles of visual and olfactory cues in pollinator attraction were tested by comparing the responses of pollinators to inflorescences subjected to three experimental treatments (intact, all bracts removed, and capitulum removed) within the natural population.

Important Findings
For fruit set, *C. capitata* is entirely dependent on pollinators, with a bee, *Anthophora* sp., being the main pollinator. Bracts present high colour distance and green contrast against the leaves. Twelve volatile compounds in the floral scent were detected, most of which have previously been reported to be attractive to a broad spectrum of bee species. Behavioural bioassays showed that both, visual cues alone and olfactory cues alone, are attractive to pollinating bees. However, visual cues alone attracted significantly more approaches than olfactory cues alone, while olfactory cues alone elicited a significantly higher landing percentage than visual cues alone. The finding suggests that, in the *C. capitata–Anthophora* sp. interaction, visual cues are mainly used for location from long distances, while olfactory cues mainly aid landing from short distances. Our results indicate that different modalities of floral cues should be considered together to understand fully the communication between flowering plant and pollinators.

Keywords: bract, floral scent, olfactory cues, spectral reflectance, visual cues

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INTRODUCTION

Plant–pollinator relationships may be one of the most ecologically important classes of plant–animal interactions, given that the majority of flowering plants (ca. 87%) are pollinated by insects or other animals (Ollerton *et al.* 2011). A frequent adaptive problem in plant and pollinating insect interactions is how partners encounter one another in a species-rich landscape (Spencer 1988). For sexual reproduction, the flowers of angiosperms have evolved amazingly diverse signalling strategies to attract pollinators; these strategies include colour patterns, structural morphologies, scented floral parts and insect

pheromones, advertising the location and amount of rewards on offer (Sakai 2002).

Visual displays are among the most important floral signals (Menzel and Shmida 1993; Schaefer *et al.* 2004; Yang *et al.* 2015; Zhang *et al.* 2015). Most of these displays are associated with the corolla. In some cases, however, floral displays are expanded, involving showy secondary structures associated with flowers but not part of the flowers themselves, such as the large translucent cream-coloured bracts covering the whole raceme in *Rheum nobile* (Polygonaceae) and the large white bracts surrounding the capitulum in *Davidia involucrate* (Nyssaceae) (Sun *et al.* 2008; Song *et al.* 2013). These bracts contrast strongly with surrounding leaves, and they have been suggested, act like flags, increasing the distance over which pollinators can detect the flowers (Schaefer *et al.* 2004). Several studies have shown that experimental clipping of the conspicuous bracts from inflorescences or flowers significantly reduces pollinator visits (Borges *et al.* 2003; Herrera 1997; Keasar *et al.* 2009; Sun *et al.* 2008; Song *et al.* 2013).

Olfactory cues are also very important in pollinator attraction. Floral scents are complex mixtures of volatile organic compounds emitted by floral tissues (Knudsen *et al.* 1993; Pellmyr and Thien 1986). Quantitative and qualitative variations in these floral compounds offer a virtually infinite number of combinations, making floral scent one of the most variable aspects of plant phenotype (Knudsen and Gershenzon 2006). Generally, insects have many different olfactory receptors, e.g. honeybees have approximately 170 olfactory receptors, enabling them to distinguish different combinations of scent compounds (Robertson and Wanner 2006). Thus, pollinating insects can distinguish between different plant species as well as between rewarding and nonrewarding flowers of the same species (Chen *et al.* 2009; Kunze and Gumbert 2001; Proffit *et al.* 2008; Song *et al.* 2014).

Compared to visual cues, it has been hypothesized that olfactory cues play a more important role in indicating specific host plant species, in signalling the appropriate phenological stage for pollinator visits and in attracting the pollinator towards the rewardless sex in dioecious plant species (Chen *et al.* 2009; Hossaert-McKey *et al.* 2010; Song *et al.* 2014). However, examples in which the attractiveness of floral colour is more important than fragrance are also known, especially for some flower mimicry systems (Galizia *et al.* 2005; Roy and Raguso 1997). In some other systems, the combination of visual and olfactory cues is thought to be essential for flower-finding and recognition by pollinators (Burger *et al.* 2010; Kulahci *et al.* 2008; Kunze and Gumbert 2001). So far, however, with only a few exceptions, the roles of visual and olfactory cues have been quantified separately. In particular, when one stimulus is more striking than the other one, the roles of the latter are usually ignored. For example, for plants with showy extra-floral displays, olfactory signals have received relatively less attention compared with visual signals (Borges *et al.* 2003; Keasar *et al.* 2009; Sun *et al.* 2008).

Cornus capitata is a medium-sized tree that is native to East Asian and popular as an ornamental plant. It is an obligate out-crossing species and depends entirely on insects for pollination (Song *et al.*, unpublished data). A striking feature of *C. capitata* is that the base of each capitulum is surrounded by four large, petaloid, white bracts during anthesis, providing a spectacular visual display (at least to humans), while each floret is rather small and inconspicuous (Fig. 1a). Thus, we postulated that the bracts of *C. capitata* might play an important role in pollinator attraction. Although *C. capitata* flowers are generally perceived to lack distinguishing scents, a recent

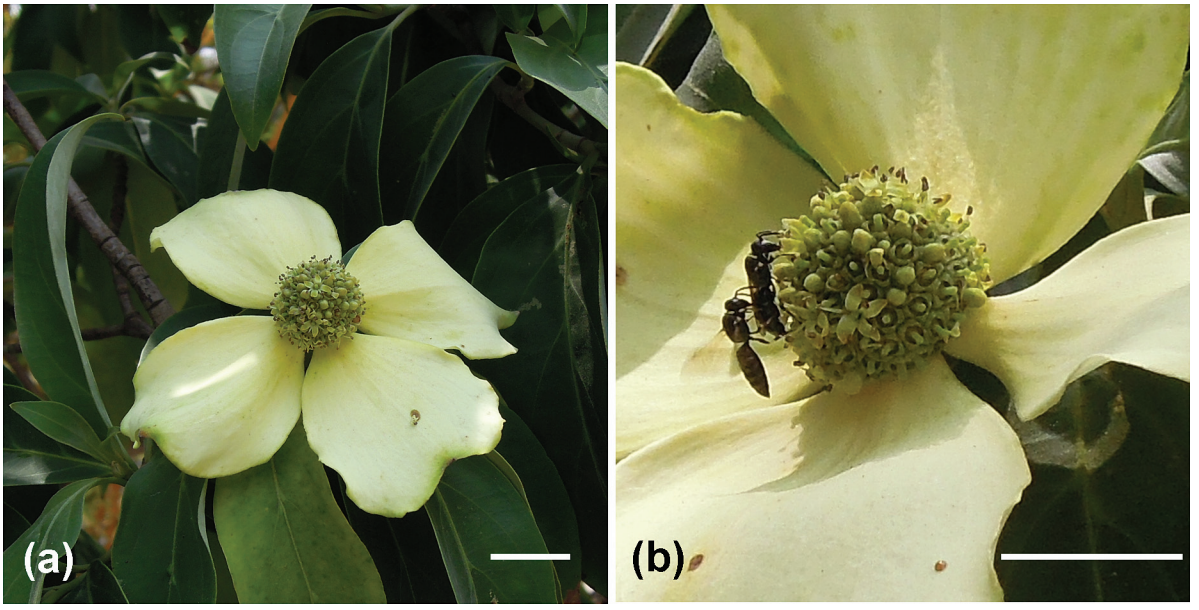

Figure 1 : (**a**) An inflorescence of *Cornus capitata*; (**b**) two *Anthophora* bees visiting the flowers. Bars: 2 cm.

study showed that another species in this genus, *C. florida*, the flowers of which are also considered scentless to humans, emitted a large number of volatile compounds that may be involved in attracting pollinators in the wild for cross pollination (Zhuang *et al.* 2008). Thus, we postulated that flowers of *C. capitata* may emit volatiles and that these volatile compounds may also be important for pollinator attraction. In this study, we characterized the visual and olfactory flowering signals of *C. capitata* and determined their roles in pollinator attraction. We aimed to answer the following questions: (i) Do the bracts contrast with the leaves in colour? If so, does the visual signal affect pollinator behaviour in the absence of an olfactory cue? (ii) Do the flowers emit volatile compounds? If so, does the floral scent affect pollinator behaviour in the absence of any visual cue? (iii) What are the relative roles of the visual and olfactory cues in attracting pollinators?

MATERIALS AND METHODS

Study species and sites

Cornus capitata (Wall.) Hutch (Cornaceae) is a deciduous tree growing up to 15 m in height, found in woodlands at altitudes of 1300–3150 m. Inflorescences are composed of 80–100 perfect flowers contained within a capitulum 1.8 cm across that is typically subtended by four large and showy white bracts, which reach their final size (4.19±0.04 cm × 3.07±0.04 cm) when the flowers open, and persist until 5–7 days after anthesis (Fig. 1a). *C. capitata* flowers have four small (3 mm long) petals inserted into a green tubular calyx (1 mm long). Each flower secretes minute amounts of nectar. The plant flowers from early May to mid-June; single flowers last 1–2 days and the whole capitulum 3–4 days.

This study was conducted at two sites: Hamaze Mountain (25°05′44″N, 102°46′15″E, 1963 m a.s.l.) and Kunming Botanical Garden (25°08′42″N, 102°44′31″E, 1788 m a.s.l.), in Kunming City, Yunnan Province, Southwest China.

Pollinator observations and pollination experiments

Floral visits were observed from 09:00 to 18:00 h over five successive days at both sites in 2014. During one night of observation, no pollinators were seen. Insect visitors were collected and sent to the Xishuangbanna Tropical Botanical Garden of the Chinese Academy of Sciences for identification. In addition, the behaviour of visitors on inflorescences was observed and recorded.

We used the method of Yuan *et al.* (2008) to test whether wind plays a role in the pollination of *C. capitata*. In the Hamaze Mountain population, a single plant (3 m in height) was chosen and microscope slides covered with petroleum jelly were placed around this plant at 0.5 m intervals, 2.5–3.0 m above the ground, to a distance of 10 m away. These slides were collected after 2 days and brought back to the laboratory for the captured pollen grains to be counted under a light microscope. To determine whether pollination of *C. capitata* depends on pollinators, two pollination experiments were conducted in the Hamaze Mountain population: (i) natural pollination: 30 capitula were randomly selected and these capitula were not manipulated; (ii) autonomous self-pollination: 30 capitula were selected randomly and these capitula were bagged with nylon mesh bags throughout their flowering period to excluded pollinating insects.

Visual cues

Colour measurements

Thirty leaves and bracts, respectively, were randomly collected from Hamaze Mountain and their reflectance spectra between 300 and 700 nm at 5 nm intervals were measured relative to a magnesium sulphate, highly ultraviolet-reflecting white standard at an angle of 45° (thus minimizing surface reflection). The leaves and bracts were kept fresh until they were measured (within 2 h) using a spectroradiometer (USB Ocean Optics 2000+) equipped with a Xenon Pulse X2 lamp light source, following Niu *et al.* (2014).

Behavioural bioassays

After identifying the visitors to flowers of *C. capitata*, the main pollinator was used for behavioural bioassays as follows (as it was for the olfactory behavioural bioassays and the field experiments described below). In order to test the roles of visual cues in attracting pollinating bees, we performed a dual-choice bioassay in a glass box (3.5×2.0×0.8 m; length × width × height) set up in a greenhouse. Two identical quartz glass jars (20 cm diameter, 20 cm height) were placed at one end of the box, 1 m away from each other. These jars contained the various treatments used in the visual tests and allowed the bees tested to see a particular treatment but not to perceive it via olfactory cues (Dötterl *et al.* 2014). We performed the following five comparisons: (i) inflorescences with intact bracts versus empty jar; (ii) inflorescences with bracts removed versus empty jar; (iii) inflorescences with bracts replaced by leaves versus empty jar; (iv) inflorescences with intact bracts versus inflorescences with bracts removed; (v) inflorescences with intact bracts versus inflorescences with bracts replaced by leaves. The last four comparisons enabled us to determine whether capitula play a visual role in pollinator attraction. The artificial bracts made of leaves were similar in shape and size to the natural bracts. In these experiments, six inflorescences were enclosed in each jar. At the start of each test, one bee was placed at the other end of the box (the distance to both jars was the same–c. 3 m), and their behaviour was recorded for 5 min. The responses of the bees were classified into two categories: (i) bees landed on either of the two treatment jars within 5 min; (ii) bees made no choice within 5 min. Tests were repeated 25 times and each bee was used for only a single test. All tests were performed on sunny days when the temperature was at least 20°C and between 11:00 h and 15:00 h. The location of the treatments was alternated every five tests to avoid position effects. The inflorescences enclosed in the jars were replaced with new ones after 10 bees had been tested. All inflorescences and bees used for the behavioural tests were collected on *C. capitata* in the Hamaze Mountain population (as was the case for the olfactory behavioural bioassays below).

Olfactory cues

Collection and identification of floral scent

Floral volatiles emitted from *C. capitata* were collected using the dynamic headspace adsorption method (Chen *et al.* 2009; Song *et al.* 2014). Inflorescences were collected from Hamaze Mountain at 10:30 h and the cut ends were wrapped in moist cotton to avoid dehydration. Six inflorescences were placed in single glass chambers (15 cm in diameter and 20 cm in height). Charcoal-purified air was introduced into the chamber through the entrance by a battery-driven air pump (Qihai Machinery & Electric Co., Ltd, Chengdu, China) and a glass cartridge (7 mm internal diameter) filled with 100 mg of Porapak Q adsorbent (mesh 60/80, Waters Associates, Inc., Milford, MA, USA) was connected to the exit of the chamber; the air flow rate was 400 ml min^{-1}. Collection was conducted for 4 h on a sunny day between 11:00 h and 15:00 h, and repeated five times. During collection of the volatiles, controls (empty chambers) were used to check for any environmental contamination. One internal standard, 3000 ng *n*-nonane was added to each sample to estimate the total scent emission.

Compounds in the samples of volatiles were identified using a gas chromatography–mass spectrometry (GC-MS) system (Agilent HP6890GC/5973MS) equipped with a HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}, and the injector temperature was set to 250°C. The column temperature began at 40°C and was increased to 250°C at a rate of 3°C min^{-1}. Compounds were identified based on automated matching of the mass spectra with Wiley7n.1 libraries or comparison of the retention data with published data.

Behavioural bioassays

In order to test the role of olfactory cues produced by inflorescences of *C. capitata* in attracting pollinating bees, dual-choice glass Y-tube olfactometers (stem 10 cm; arms 15 cm, at 55° angle; inner diameter 2.5 cm) were used; in these, only chemical attraction was possible (Chen *et al.* 2009). Purified air was blown into the tube by a battery-driven air pump at a rate of 400 ml min^{-1}. The two arms of the Y-tube were connected to a glass container containing an odour source. A single bee was placed at the entry to the Y-tube, and given 5 min to respond. The responses of the bees were classified into two categories: (i) bees walked into one of the two arms of the olfactometer within 5 min; (ii) bees did not respond within 5 min. Tests were repeated 30 times and each bee was used for only a single test. Treatment combinations used were: (i) scent emitted by intact inflorescences versus purified air; (ii) scent emitted by intact inflorescences versus scent emitted by inflorescences with capitula removed; (iii) scent emitted by inflorescences with capitula removed versus purified air; (iv) scent emitted by inflorescences with bracts removed versus purified air; (v) scent emitted by inflorescences with bracts removed versus scent emitted by intact inflorescences. The last four comparisons enabled us to determine whether olfactory cues responsible for pollinator attraction are mainly produced by capitula. Experiments were conducted on sunny days with an air temperature of at least 20°C, between 11:00 h and 15:00 h. The treatment locations were alternated every five tests to avoid position effects. The inflorescences enclosed in the glass containers were replaced with new ones after 10 bees had been tested.

Relative roles of visual and olfactory cues

To determine the relative roles of visual and olfactory cues in attracting pollinating bees, we recorded bee visits to three kinds of inflorescence: (i) intact; (ii) all bracts removed; (iii) capitulum removed. Each day before the observations, one inflorescence on each of 15 flowering stems on a single *C. capitata* individual was selected randomly and other inflorescences on these flowering stems were removed. Thus, there were no other inflorescences within at least 1 m of the inflorescences selected. The 15 inflorescences selected were assigned to the aforementioned three treatments. During these observations, we counted the number of bee approaches (i.e. approaching the inflorescence at a maximum distance of 5 cm, because the bees usually hover at this distance away from the inflorescence before landing on it; author's personal observation). Meanwhile, we recorded whether the bees that had approached landed and then calculated landing percentage as the number of landing bees/number of approaching bees (Lunau 1992). Observations were carried out at 9:00, 12:00, 15:00 and 18:00 h by three observers simultaneously. Each observation period for each inflorescence lasted for 30 min. Experiments were conducted on four sunny days in the Hamaze Mountain population. In total, 80 30-min observations on 20 inflorescences per treatment were conducted. For the landing percentage, 4, 42 and 8 observations with no bee approaches recorded for treatments 1, 2 and 3, respectively, were excluded from the analysis.

Data analysis

Data obtained from spectral measurements were used to calculate colour distances and green contrast between the two samples based on the trichromatic vision of bees, following Chittka (1992) and Giurfa *et al.* (1996). Spectral sensitivity functions for the bees were obtained from Peitsch *et al.* (1992). Results obtained from dual-choice behavioural bioassays were analysed using chi-square tests to determine whether bees were attracted to one or two experimental treatments. Kruskal–Wallis test was employed to compare the responses of bees to inflorescences subjected to three experimental treatments in the field, followed by post hoc analysis (Mann–Whitney *U* test). All analyses were performed using SPSS 18.0 (SPSS Company, Chicago, IL, USA), with measured variables presented as mean ± SE.

RESULTS

Pollinator observations and pollination experiments

Flowers of *C. capitata* were visited by six insect species during our field observation period (Table 1). The most frequent visitor at both sites was *Anthophora* sp. (Apidae, Hymenoptera) (Fig. 1b).

Table 1: insect visitors captured or observed on the flowers of *Cornus capitata* at Kunming Botanical Garden and Hamaze Mountain

	Kunming Botanical Garden		Hamaze Mountain	
Taxon	Total number of visits	Percentage of total visits	Total number of visits	Percentage of total visits
Hymenoptera				
Anthophora sp.	701	91.3	1027	88.1
Diptera				
Eristalis arbustorum	38	4.9	84	7.2
Coleoptera				
Themus imperialis	4	0.5	24	2.1
Oenopia formosana	7	0.9	6	0.5
Lepidoptera				
Acraea issoria	18	2.4	21	1.8
Spindasis syama	0	0	4	0.3

In fact, of 768 and 1166 visits observed in Kunming Botanical Garden and Hamaze Mountain, 91.3 and 88.1%, respectively, were by *Anthophora* sp. The bees tended to move from one flower to another within a capitulum before moving to another capitulum, staying within a capitulum for 20.6 ± 1.3 s (n = 20), suggesting that this bee species is the most effective pollinator of *C. capitata*.

Although 0.6 ± 0.08 (n = 10) pollen grains of *C. capitata* were captured on the microscope slides (14 cm^2) coated with petroleum jelly, at a distance of 0.5 m from the source, no pollen grains were captured at 1 m or more from the source; this suggests that wind pollination plays a negligible role in the pollination of *C. capitata*. Capitula covered with nylon bags produced no fruit (n = 30), while fruit set resulting from natural pollination was 30.8 ± 0.7 % (n = 30), suggesting that insect pollination is necessary for fruit set.

Visual cues and behavioural bioassays

The spectral reflectance measurements revealed that the reflectance of the bracts is significantly different to that of the leaves (Fig. 2). The colour distance and green contrast between bracts and leaves is 0.26 units and 72.9%, respectively.

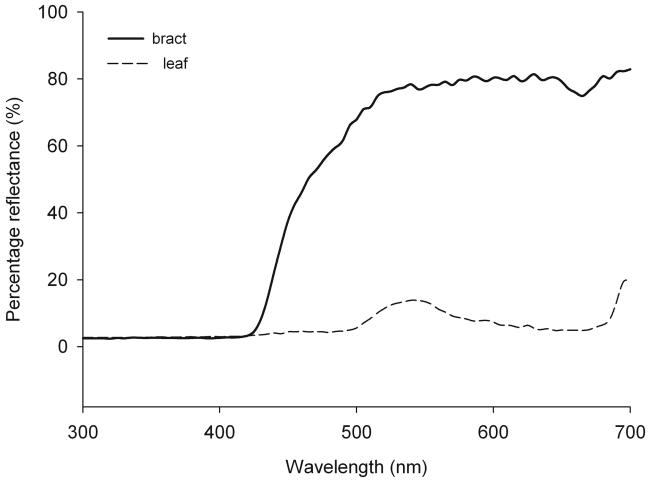

Figure 2: spectral reflectance of bracts and leaves of *Cornus capitata*.

When inflorescences with bracts removed or bracts replaced by leaves were presented as an alternative to an empty jar, very few *Anthophora* bees were stimulated to respond (only 4 and 8 out of 25 bees, respectively) and those that did respond did not actively favour one treatment over the other (Table 2(a, b)), suggesting that the inconspicuous capitulum alone has no role in pollinator attraction via its visual display. However, bees showed a strong preference for inflorescences with intact bracts when given a choice between these and an empty jar or inflorescences with bracts removed or inflorescences with bracts replaced by leaves (Table 2(c–e)).

Olfactory cues and behavioural bioassays

In the floral scents emitted by *C. capitata*, we found 12 different compounds at detectable levels (Table 3). The average total amount of volatiles emitted per inflorescence per hour was 367.8 ± 36.2 ng (n = 5).

Anthophora bees showed a strong preference for intact inflorescences when given a choice between these and purified air or inflorescences with capitula removed (Table 4(a, b)), but exhibited no preference between inflorescences with capitula removed and purified air (Table 4(c)). When inflorescences with bracts removed were presented as an alternative to purified air, a high proportion of bees were attracted to inflorescences with bracts removed (Table 4(d)). However, when inflorescences with bracts removed were presented as an alternative to intact inflorescences, bees did not favour one signal over the other (Table 4(e)).

Relative roles of visual and olfactory cues

Visual cues alone attracted significantly more approaches (3.05 ± 0.21) than olfactory cues alone (0.98 ± 0.14), but significantly fewer approaches than the combination of both visual and olfactory cues (5.88 ± 0.33) (χ^2 = 107.7, df = 2, P < 0.001; Fig. 3a). However, olfactory cues alone elicited a significantly higher landing percentage (62.28 ± 5.79%) than visual cues alone (4.85 ± 1.36%), but not than the combination of both visual and olfactory cues (55.7 ± 1.88%) (χ^2 = 115.4, df = 2, P < 0.001; Fig. 3b).

Table 2 : visual attraction of *Anthophora* sp. to inflorescences of *Cornus capitata*

Comparison type	Treatment		Results				
	A	B	No. of bees	NC	A	B	Chi-square (*P*)
a	−B	E	25	21	1	3	0.317
b	−B+L	E	25	17	3	5	0.480
c	+B	E	25	2	21	2	<0.001
d	+B	−B	25	3	18	4	0.003
e	+B	−B+L	25	1	21	3	<0.001

Choices made by bees presented with paired combinations of: inflorescences with bracts removed (−B), inflorescences with bracts replaced by artificial bracts made of leaves (−B+L), inflorescences with intact bracts (+B) and empty jars (E). Treatments A and B indicate the two visual cues available; NC indicates the number of bees that made no choice within 5 min; Results A and B indicate the number of bees that actively chose A or B, respectively. The Chi-square tests compared: where a choice was made, the choice of one cue over the other; the values (*P*) are the probability of each test result. All results were compared to a theoretical ratio of 0.5:0.5.

Table 3 : relative amounts of volatile compounds emitted by *Cornus capitata* inflorescences at anthesis

	Compounds	Relative amount (%)
1	4-ethylbenzoic acid	26.42±4.98
2	Tetradecane	12.79±0.59
3	4-hydroxy-4-methyl-2-pentanone	9.95±1.11
4	Ketoisophorone	9.00±1.19
5	E.E-α-farnesene	8.27±2.31
6	Furan-2-carboxaldehyde	7.29±0.36
7	Nonanal	6.39±0.64
8	Decanal	5.98±0.14
9	Benzaldehyde	5.20±0.47
10	E-β-ocimene	3.78±0.65
11	4,8-dimethyl-1,3,7-nonyl triene	2.59±0.39
12	Linalool	2.33±0.36

Relative amounts indicate the proportion of each compound in the entire bouquet (%) (n = 5).

DISCUSSION

Visual cues

It has long been claimed that non-floral showy structures, including bracts, improve pollination success by enhancing visual displays (Herrera 1997). In this study, the mean number of bee approaches decreased by 83% for inflorescences with bracts removed as compared to intact inflorescences (Fig. 3). Similarly, in the dual-choice bioassays, when we presented pollinators with inflorescences with either bract removed or with bracts replaced by leaves, *Anthophora* bees preferentially visited inflorescences with intact bracts. Clearly, our results indicate that the large white bracts of *C. capitata* play an important role in pollinator attraction. This is in agreement with previous reports on the relationship between visual advertisements by plants and pollinator visits (Borges *et al.* 2003; Keasar *et al.* 2009; Sun *et al.* 2008; Song *et al.* 2013). One possible interpretation of these findings is that the bracts of *C. capitata* increase the visibility of inflorescences to pollinators. Spectral measurements revealed that the bracts of *C. capitata* contrast strongly with the leaves in all spectral ranges except the ultraviolet. The hexagon distance between the bracts and leaves is 0.26 units, and bees are known to discriminate well between colours with hexagon distances > 0.1 units (Dyer and Chittka 2004), suggesting that *Anthophora* bees can perceive this difference. Giurfa *et al.* (1996) suggested that colour distance was not the only optical parameter affecting target detection. When the visual angle (α_{min}) subtended by a target is small (less than 15°, but at least 5°), which is associated with a greater distance or a smaller target, detection by bees depends on green contrast (Spaethe *et al.* 2001). In this study, the bracts of *C. capitata* produced 72.9% green contrast against the background, which is above the minimum value (7.2%) for target detection at small visual angles (α_{min}=5°) (Kaiser and Liske 1974). According to Giurfa *et al.* (1996), the farthest distance from which an intact inflorescence of *C. capitata* (c. 10 cm in diameter) would be detectable is about 115 cm at best, and 23 cm for an inflorescence with only the capitulum, supposing it could be detected at all. Thus, the high colour distance and green contrast in combination with the increase in size produced by bracts make inflorescences of *C. capitata* detectable at greater distances. Furthermore, inflorescences of *C. capitata* generally occur in clusters, which may further increase the range over which they can be detected by bees (Kevan *et al.* 1990). In addition, *C. capitata* generally grows in high light habitats where visual cues are most exploitable (Borges *et al.* 2003). Consequently, these bracts may make the inflorescences stand out from the surrounding foliage, functioning as ‘detective cues’ (Borges *et al.* 2003).

Olfactory cues

Floral scents have been identified as key signals for elaborate communication in a wide variety of plant–insect interactions (Hossaert-McKey *et al.* 2010; Knudsen *et al.* 1993). In our dual-choice bioassays, in which only olfactory attraction was possible, most bees were attracted to intact inflorescences when tested against purified air (Table 4), suggesting that pollinating bees can be attracted by the floral scent emitted by *C. capitata*.

Table 4 : chemical attraction of *Anthophora* sp. to inflorescences of *Cornus capitata*

	Treatment		Results				
Comparison type	A	B	No. of bees	NC	A	B	Chi-square (*P*)
a	+C+B	PA	30	4	24	2	<0.001
b	+C+B	−C+B	30	2	23	5	<0.001
c	-C+B	PA	30	16	6	8	0.593
d	+C-B	PA	30	5	22	3	<0.001
e	+C−B	+C+B	30	3	12	15	0.564

Choices made by bees presented with intact inflorescences (+C+B), inflorescences with capitula removed (−C+B) and inflorescences with bracts removed (+C−B) against purified air (PA), and inflorescences with bracts removed and inflorescences with capitula removed against the scent emitted by intact inflorescences. Treatments A and B indicate the two available olfactory cues; NC indicates the number of bees that made no choice within 5 min; Results A and B indicate the number of bees that actively chose A or B, respectively. The Chi-square tests compared: where a choice was made, the choice of one cue over the other; the values (*P*) are the probability of each test result. All results were compared to a theoretical ratio of 0.5:0.5.

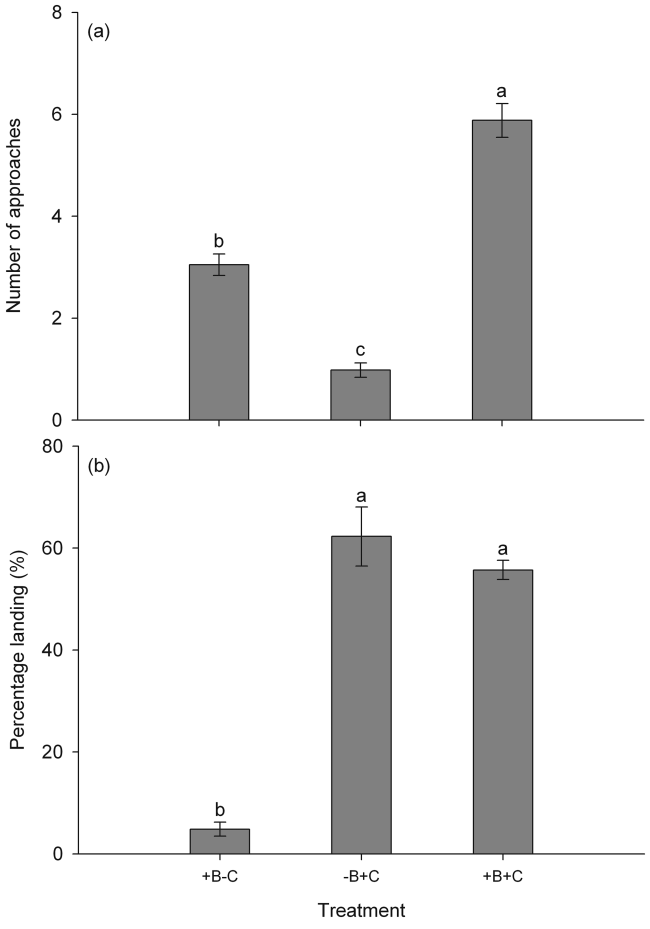

Figure 3: (**a**) Mean (± SE) number of *Anthophora* bees that approached *Cornus capitata* inflorescences subjected to three experimental treatments during an observation period of 30 min; (**b**) Mean (± SE) percentage landing of *Anthophora* bees on *C. capitata* inflorescences subjected to three experimental treatments after approaching. Different letters denote significant differences at $P < 0.05$. *+B−C*: inflorescences with capitula removed; *−B+C*: inflorescences with bracts removed; *+B+C*: intact inflorescences.

This was also validated by our field experiments: removal of olfactory cues by removing capitula resulted in approaches by bees dropping by 48% compared to approaches to intact inflorescences (Fig. 3). The role of olfactory cues in the reproduction of scented plants, which can be detected by humans, is easy to understand. However, their role in the reproduction of plants that are scentless to humans is elusive. Our analysis of volatiles showed that *C. capitata* flowers emitted a number of floral compounds (Table 3) that may be detected by insects but not humans (Kaupp 2010). Of the 12 compounds identified in the floral scent of *C. capitata*, five (E-β-ocimene, linalool, nonanal, ketoisophrone and decanal) were also recorded from *C. florida* (Zhuang *et al.* 2008). Most of these compounds, such as the linalool, E.E-α-farnesene, E-β-ocimene and benzaldehyde, have been shown to be attractive to a broad spectrum of bee species (Blight *et al.* 1997; Borg-Karlson *et al.* 2003; Granero *et al.* 2005; Henning *et al.* 1992). Thus, using human olfaction alone is not sufficient for judging the role of olfactory cues in plant reproduction. Analysis of a dissected inflorescence of *C. florida* showed that the major volatile constituents were primarily emitted from the florets (Zhuang *et al.* 2008). Although we did not determine precisely which parts of the inflorescences emitted the volatiles responsible for pollinator attraction, our dual-choice bioassays suggested that these volatiles are mainly emitted by the capitula, because inflorescences with only capitula and no bracts were also attractive. In communication between flowering plants and pollinators, the chemical messages responsible for pollinator attraction can be in the form of either a few specific chemical substances or combinations of common compounds in certain proportions (Svensson *et al.* 2010). Thus, further studies examining the responses of bees to pure and synthetic volatile compounds individually or in combination, should be conducted to determine the key constituents associated with pollinator attraction.

Relative roles of visual and olfactory cues

In our field experiments, the number of bees approaching the inflorescences with only olfactory cues was significantly lower than those approaching inflorescences with only visual cues; this suggests that visual cues are initially more important than olfactory cues for orientation, as reported in most studies on honey bees and bumble bees (Burger *et al.* 2010; Dobson 1991; Galen and Kevan 1980). In fact, given

the considerable influence that the environment has on the dispersal of volatiles and interference caused by the complex volatile background from natural and anthropogenic resources, it is often suggested that visual cues are more effective than olfactory ones for long-distance signalling (Riffell *et al.* 2014; Schaefer *et al.* 2004). However, we cannot rule out the possibility that olfactory cues might have a function in orientation, since some *Anthophora* bees approached the inflorescences when only olfactory cues were offered, in the same way that has been shown for the *Macropis* bees, which were attracted by the floral scent from a distance of several meters (Dötterl and Schäffler 2007). It has been reported that olfactory cues are essential in eliciting landing behaviour by some bee species (Giurfa *et al.* 1995). In our study, however, a few bees did land when visual cues alone were offered. Although our results do not rule out the possibility that visual cues play a role in inducing the bees to land, the landing percentage was significantly reduced when olfactory cues were removed, suggesting that olfactory cues are more important than visual cues for landing once the inflorescence has been found. In fact, unlike the bracts of many other plant species, such as *D. involucrate* (Sun *et al.* 2008), the bracts of *C. capitata* do not wither soon after anthesis and provide no obvious cues to the developmental stage of the flowers (there is no significant difference in morphology and reflectance between anthesis and post-anthesis; Song *et al.*, unpublished data), which may also indicate that the visual cues of the bracts mainly function for orientation and are not specific for the particular inflorescence, while the floral scent is mainly responsible for landing behaviours. These results are consistent with earlier studies involving other plant species and other bee species (Burger *et al.* 2010). However, in some bee species, it has been found that long-distance location depends on olfactory cues and short-distance landing is induced by visual cues (Galizia *et al.* 2005). Given the high diversity of bees, it is not surprising that the relative roles of visual and olfactory cues vary among species.

In conclusion, our study shows that there are both visual and olfactory components in attracting the main pollinator, *Anthophora* sp., to the inflorescences of *C. capitata*, but the relative importance of these cues varies between stages for each visitation event: visual cues are initially more important than olfactory cues for location, while olfactory cues are more important than visual cues for landing. These results indicate that different modalities of floral cues should be considered in a combined approach in order to understand fully the communication between flowering plants and pollinators. For many plants, floral scent emission is a dynamic process, with peak emission generally coinciding with the most active visitation period for the pollinators (Patt *et al.* 1995). Because the observed predominant pollinator of *C. capitata*, *Anthophora* sp., has a diurnal lifestyle, comparative studies of the timing of the peak emission of volatiles and the timing of maximum pollinator activity would be particularly useful for understanding the role of floral scent in pollinator attraction more precisely.

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